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Herpetofauna from a high altitude remnant in the Atlantic Forest

HERPETOFAUNA IN A HIGH ALTITUDE ATLANTIC FOREST REMNANT FROM SOUTHEASTERN BRAZIL

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Abstract. Human activities have significantly impacted the Atlantic Forest, posing serious threats to its biodiversity. Notable gaps remain in our knowledge of wildlife inhabiting its higher montane regions, which are particularly important due to their unique biota. Investigating biodiversity in these high-altitude areas is especially critical for taxa that are dependent on water-related habitats and those that are vulnerable to habitat loss and the effects of climate change, such as the ectothermics. Our study surveyed amphibian and squamate reptiles, providing notes on their natural history and morphology within a 3,600-hectare fragment of the Brazilian Atlantic Forest in the Serra do Mar mountainous region. The area is located within the municipality of Nova Friburgo, in the state of Rio de Janeiro, and encompasses elevations ranging from 1,000 to 2,000 meters. The fieldwork employed 552 hours of visual encounter surveys, 960 bucket-day efforts for pitfall traps with drift fences, and opportunistic sampling methods. We found 317 specimens, comprising 21 species of amphibians and 16 of squamate reptiles. The amphibians represented nine families, including 18 species endemic to the Atlantic Forest and three to Rio de Janeiro. The reptile assemblage encompassed five families, with nine species endemic to the Atlantic Forest. These findings highlight the region's importance for maintaining biodiversity and highlight the need for further studies to support conservation actions to prevent increasing anthropogenic impacts.

Keywords: Amphibians; Conservation; Diversity; Endemism; Inventory; Reptiles.

INTRODUCTION

The Atlantic Forest, a biodiversity hotspot in eastern South America with approximately 92% of its range within Brazil, hosts an extraordinary richness of species and exhibits exceptionally high levels of endemism (Ribeiro *et al.* 2011). The Serra do Mar is one of the most extensively studied centers of endemism in the Atlantic Forest (Figueiredo *et al.* 2021). Nonetheless, new occurrence records and frequent descriptions of new species (*e.g.,* Carmo *et al.* 2022) continue to demonstrate that the species richness in this region, as well as in the Atlantic Forest more generally, is greater than previously estimated (Figueiredo *et al*. 2021).

Knowledge gaps are known as “Wallacean shortfall”, when there are geographical distribution missing pieces, and "Linnaean shortfall," referring to the difference between the number of existing species and those scientifically described (Hortal *et al.* 2015). These shortfalls hinder our understanding and appropriate protection of this global biodiversity hotspot (Myers *et al.* 2000), especially in areas with high altitude gradients due to difficult access and logistics for sampling (Guedes *et al.* 2020). Although some studies conducted in high-altitude areas along the Serra do Mar have assessed amphibian species richness (*e.g.*, Siqueira *et al*. 2011; Folly *et al*. 2016), knowledge about squamate reptiles remains scarce, with several areas represented by only a few publications (*e.g.*, Barros-Filho 2008). Amphibians and reptiles often include cryptic species and species that exhibit elusive behaviors, which make these representatives particularly susceptible to information gaps (*e.g.,* Maciel *et al.* 2019). In addition to the lack of basic data on species occurrence, which poses a threat to biodiversity conservation, the biological traits inherent to herpetofauna may also increase their vulnerability. For example, the permeable skin of amphibians, along with the generally biphasic life cycle that involves aquatic eggs (Van Sluys *et al.* 2009), the reliance of reptiles on specific thermal microenvironments for optimal metabolism (Vitt & Caldwell 2009), and the limited dispersal capacity of several representatives of both groups (Inman *et al.* 2023) make these animals vulnerable to climate change and other human impacts (Vitt & Caldwell 2009).

Abiotic factors, such as temperature variation along the latitudinal and altitudinal gradients, constitute a non-living part of an ecosystem that shapes its environment and affects the life cycle and spatial distribution of amphibians and reptiles (Siqueira & Rocha 2013). The Atlantic Forest has extensive altitudinal variation, sometimes exceeding 2,000 m (Ribeiro *et al.* 2011), which can promote the isolation of populations and may lead to the development of unique evolutionary lineages and microendemic species (Rull & Carnaval 2020). Factors such as the degree of isolation, small population size, demographic stochasticity associated with climate change and anthropogenic pressures increase the susceptibility of these lineages to extinction (Foley 1994).

Organisms with low thermal tolerance and limited ability to disperse may be more prone to isolation due to altitudinal variation (Rull & Carnaval 2020). As a result, some amphibian and reptile lineages exhibit high diversity along the Atlantic Forest altitudinal gradient, displaying a high variety of habitats and microhabitats use with specific preferences (Siqueira *et al.* 2011).

Amid limited knowledge of local biodiversity and ongoing habitat loss in the Serra do Mar, field-based biotic inventories are essential for describing wildlife, informing conservation policies and ensuring the preservation of ecosystem services. This lack of baseline information poses essential challenges for effective conservation and constitutes a noteworthy knowledge gap on distribution patterns, especially in a tropical forest under intense human pressure (Diniz *et al.* 2022).

In this context, our study aims to fill the biodiversity data gaps within a mountainous remnant of the Atlantic Forest by surveying the richness and composition of amphibians and squamate reptiles in a well-conserved high-altitude portion of Serra do Mar. We leverage our *in situ* fieldwork efforts to collect and report information on species microhabitat use and morphological data whenever possible, contributing to the understanding of the natural history and taxonomy of Neotropical fauna.

MATERIAL AND METHODS

Study area

The study area is located in the São Lourenço district in the municipality of Nova Friburgo, state of Rio de Janeiro, situated in the Serra dos Órgãos, a geological portion of Serra do Mar. The local climate is classified as subtropical humid and super humid (Cfb) to tropical dry to super humid (Aw), according to the Köppen-Geiger classification (Beck *et al.* 2018). The average annual temperatures vary between 18º and 26ºC in summer and 10º to 18ºC in winter, with temperatures occasionally below 0ºC (Fick & Hijmans 2017). The average annual precipitation is approximately 2,000 mm, with the rainy season occurring from October to March and the dry season occurring from April to September (IBGE 2024).

The sample area is located on the *campus* of Instituto Vital Brazil (22º20'44.87” S and 42º37'16.75” W), located in the buffer zone of Parque Estadual dos Três Picos (PETP), with a forested area of 3,600 ha. Its altitude varies between 1,000 m and 2,000 m above sea level (Fick & Hijmans 2017) (Figure 1). Although the high-altitude forests in the São Lourenço district are considered well conserved, they may suffer from increasing edge effects due to the presence of anthropized areas in their surroundings, such as urbanization, agriculture, and grazing areas. The phytophysiognomy of the area follows Veloso *et al.* (1991) and consists of Dense Evergreen Moist Forest with large trees and a uniform canopy (at altitudes *ca.* 500 – 1,500 m), Dense Alto Montane Ombrophilous Forest with medium-sized trees and a uniform canopy (at altitudes *ca.* 1,500 - 1,800 m) and a high-altitude field environment, with rocky outcrops, grasses and small trees (above >1,800 m). There is a small area with an exotic *Eucalyptus* plantation.

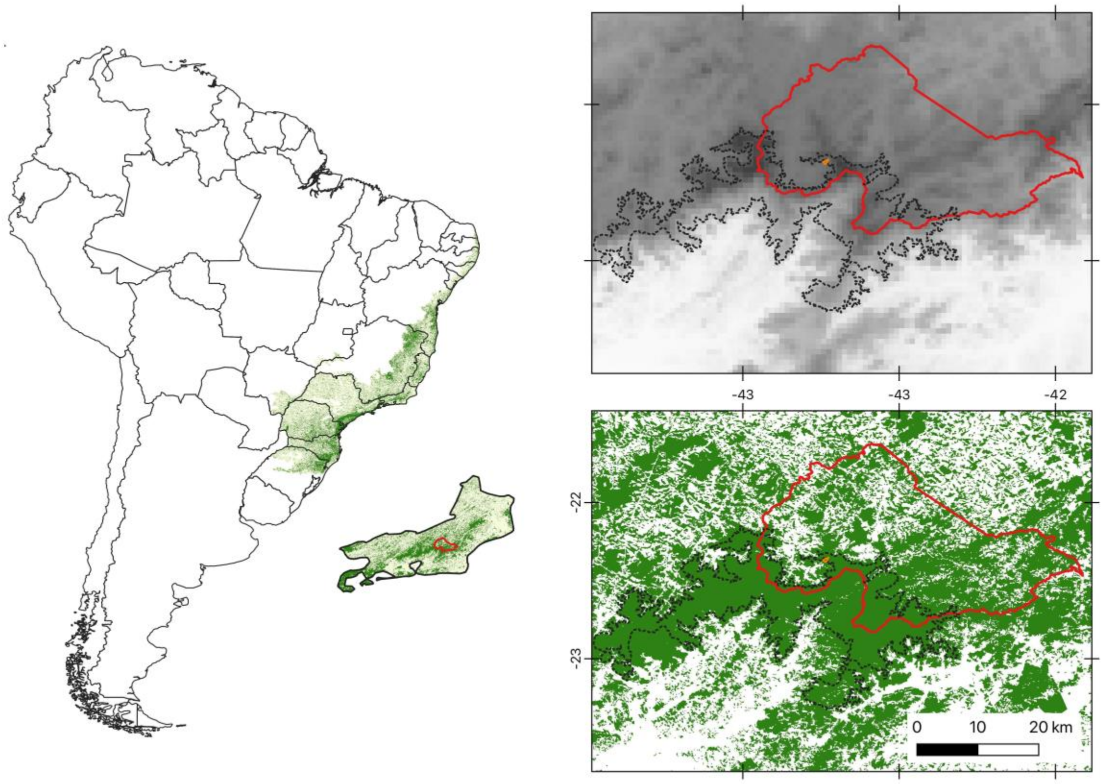

Figure 1. Map of the study area showing the municipality of Nova Friburgo, state of Rio de Janeiro, Brazil. Right-up: altitudinal variation indicated by lighter tones as lower altitudes and darker shades as higher altitudes. Right-down: Atlantic Forest remnants near the study area, in which the largest continuum is the Parque Estadual dos Três Picos. Municipal limits in red; District limits in orange; Protected area limits in dotted line. Data source: IBGE (2024); Fick & Hijmans (2017); INEA (2024) and Assis *et al.* (2019).

Herpetofauna sampling in São Lourenço

Our sampling took place from May 2015 to February 2016. We conducted three field expeditions during the dry season in April, May and July 2015 and two during the rainy season on October 2015 and February 2016, three of the four expeditions lasting ten days. The second expedition during the dry season was limited to two days of surveys due to a severe winter in July that hindered our ability to sample specimens. The specimens were collected under permit #48202-1 granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO) and #025/2015 granted by the Instituto Estadual do Ambiente (INEA).

We applied the Rapid Assessment Program for surveys (IUCN 1990), formed by a team of six researchers, using the time-limited visual encounter survey (VES) and pitfalls to assess fossorial species associated with litter. The sampling sites spanned altitudes ranging from 1,100 to 1,400 m and were determined based on optimal field logistics for trap implementation and active searches. We divided the inventory area into four sampling units, each measuring 60 by 25 meters and encompassing an area of 1,500 square meters, spaced at least 400 meters apart: VES1 (22º20'49” S and 42º37'05” W), VES2 (22º20'29” S and 42º36'52” W), VES3 (22º20'25” S and 42º36'52” W) and VES4 (22º20'16” S and 42º36'46” W). Every sampling unit was visited five times per expedition, except for the three-day expedition (see details of method in supplementary materials).

We gathered specimens as vouchers to allow accurate species identification and future molecular investigations. Tissue samples (liver/muscle) were collected from each specimen and stored in sterile microtubes containing 95% ethanol, identified with specific coded labels, and stored in a freezer (for DNA extraction) at the Tissue and DNA collection of Instituto Vital Brazil. The voucher specimens were preserved in 10% formaldehyde and stored in 70% alcohol at the Coleção Biológica Instituto Vital Brazil (IVB) (Appendix S1). Field protocols for collecting and preserving specimens and biological samples adhered to Arruda *et al.* (2024). We identified the taxa to the species level whenever feasible, using current taxonomic literature data and assessing the congruence of the specimens' diagnostic features with those previously documented (*e.g.*, Peters & Orejas-Miranda 1970; Faivovich *et al.* 2005; Passos *et al.* 2010 Breitman *et al.* 2018). Taxonomic nomenclature follows Guedes *et al.* (2023a) for squamate reptiles and Segalla *et al.* (2021) for amphibians.

We identified endemic species of the Atlantic Forest and of the state of Rio de Janeiro based on Dorigo *et al.* (2018), Rossa-Feres *et al.* (2017), Guedes *et al.* (2023a), and ICMBio

(2024). We assigned the global and national species conservation status based on IUCN (2024) and ICMBio/MMA (2018).

Measurements were taken from specimens collected and preserved in the Biological Collection of Instituto Vital Brazil this data is available in the Supplementary Material Appendix S2 and S3. We also assessed the microhabitat use of the specimens encountered during fieldwork, based on accurate on-site descriptions of the microenvironment in which they were found. Information on species habits was obtained from the literature (Izecksohn & de Carvalho 2001; Mariotto *et al*. 2022; Jackson 1978; Campbell *et al.* 2004; Marques & Sazima 2004).

We estimated the effectiveness of the sampling effort with rarefaction curves through 10,000 randomizations without replacement using only standardized methods (VES and Pitfall) and evaluated the expected richness by the Jackknife 1 and Chao 1 estimators using EstimateS 9.1. The Jackknife 1 estimator employs the number of species occurring in a single sampling unit to estimate how many species may have gone unrecorded; consequently, its sensitivity is determined by the number of samples and their variance (Magurran 2004). The Chao 1 estimator uses the number of singletons and doubletons (species represented by only one or two individuals) to estimate unobserved richness, with a focus on rare species (Magurran 2004).

RESULTS

We recorded 317 specimens of 37 species, of which 21 were Anura amphibians belonging to nine families and 13 genera (Figures 2–3), and 16 were squamate reptiles, of which three lizards belonging to two families and three genera (Figure 4) and 13 snakes belonging to three families and 10 genera (Figures 4–5). No Gymnophiona or Amphisbaena was recorded. The most abundant species were *Ischnocnema* sp. (gr. *guentheri*) (n = 99 individuals) (Figure 2) for amphibians, *Enyalius perditus* (n = 11) (Figure 4) for lizards and *Bothrops jararaca* (n = 05) (Figure 5) for snakes (Table 1).

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Figure 2. Amphibians from São Lourenço, Nova Friburgo, state of Rio de Janeiro, Brazil. A: *Ischnocnema* sp. (gr. *guentheri*) morphotype I [AF]; B: *Ischnocnema octavioi*; C: *Ischnocnema parva* [AF]; D: *Ischnocnema* sp. (gr. *guentheri*) morphotype II [AF]; E: *Rhinella icterica* [AF]; F: *Rhinella ornata*; G: *Cycloramphus eleutherodactylus* [AF]; H: *Fritziana fissilis* [AF]; I: *Boana faber*; J: *Boana polytaenia* [AF]; K: *Bokermannohyla circumdata* [AF]; L: *Dendropsophus minutus* [AF]. [AF] = endemic species to the Atlantic Forest.

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Figure 3. Amphibians from São Lourenço, Nova Friburgo, state of Rio de Janeiro, Brazil. A: *Ololygon albicans* AF, RJ; B: *Ololygon flavoguttata* AF; C: *Ololygon* cf. *obtriangulatus* AF; D: *Ololygon hiemalis* AF; E: *Hylodes pipilans* AF, RJ; F: *Hylodes pipilans* AF, RJ (intraspecific variation); G: *Megaelosia goeldii* AF, RJ; H: *Leptodactylus latrans* AF; I: *Myersiella microps* AF; J: *Proceratophrys boiei* AF (individual with anophthalmia); K: *Proceratophrys* cf. *melanopogon* AF; L: *Proceratophrys* cf. *melanopogon* AF (intraspecific variation). AF = endemic species to the Atlantic Forest, RJ = endemic species to the state of Rio de Janeiro.

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Figure 4. Squamate reptiles from São Lourenço, Nova Friburgo, state of Rio de Janeiro, Brazil. A: *Heterodactylus imbricatus* [AF]; B: *Enyalius perditus* [AF]; C: *Urostrophus vautieri*; D: *Chironius bicarinatus*; E: *Atractus zebrinus*; F: *Dibernardia affinis* [AF]; G: *Dibernardia persimilis* [AF]; H: *Oxyrhopus clathratus* [AF]; I: *Dipsas alternans* [AF]; J: *Dipsas neuwiedi* [AF]; K: *Dryophylax nattereri*; L: *Echinanthera cephalostriata.* Scale bar: 1 cm. [AF] = endemic species to the Atlantic Forest.

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Figure 5. Squamate reptiles from São Lourenço, Nova Friburgo, state of Rio de Janeiro, Brazil. II.; A: *Elapomorphus quinquelineatus* [AF]; B: *Tomodon dorsatus*; C-D: intraspecific variation in *Bothrops fonsecai* [AF]; E-F: intraspecific variation in *Bothrops jararaca* [AF].. Scale bar: 1 cm. [AF] = endemic species to the Atlantic Forest.

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Table 1. Amphibians and squamate reptiles recorded at the São Lourenço sampling sites, Nova Friburgo, Rio de Janeiro, Brazil. Endemism: AF = endemic species of the Atlantic Forest, RJ = endemic species of the state of Rio de Janeiro. Sampling methods: VES = time-limited visual encounter survey, PF = pitfall and OE = occasional encounter. Conservation status: LC = least concern, according to IUCN (2024) and ICMBio/MMA (2018).

Taxon	Sampling method	Range (m)	Microhabitat	Abundance (n)	Conservation status	Endemic
AMPHIBIA (Anurans)						
Brachycephalidae						
Ischocnema sp. (gr. *guentheri*)	VES,PF,OE	1119–1272	Leaf litter	99		AF
Ischnocnema octavioi (Bokermann, 1965)	PF	1122	-	1	LC, LC	
Ischnocnema parva (Girard, 1853)	VES,OE	1100–1400	Leaf litter	3	LC, LC	AF
Bufonidae						
Rhinella ornata (Spix, 1824)	VES, PF	1161	Leaf litter/ Swamp area	26	LC, LC	AF
Rhinella icterica (Spix, 1824)	VES,PF,OE	1100–1400	Leaf litter	30	LC, LC	AF
Cycloramphidae						
Cycloramphus eleutherodactylus (Miranda-Ribeiro, 1920)	PF	1278	Leaf litter	1	LC, LC	AF
Hemiphractidae						
Fritziana fissilis (Miranda Ribeiro, 1920)	VES	1161	Bromeliad	1	LC, LC	AF
Hylidae						
Boana faber (Wied-Neuwied, 1821)	VES,OE	1100–1400	Tree trunks	4	LC, LC	
Boana polytaenia (Cope, 1870)	VES,OE	1100–1400	Shrubs	10	-, LC	AF
Bokermannohyla circumdata (Cope, 1871)	VES	1330	Tree trunks	1	LC, LC	AF
Dendropsophus minutus (Peters, 1872)	VES	1100–1400	Swamp area	1	LC, LC	AF
Ololygon albicans Bokermann, 1967	OE	1100–1400	Swamp area	2	LC, LC	AF, RJ
Ololygon sp. (catharinae complex)	VES	1153–1161	Swamp area	1		
Ololygon hiemalis (Haddad and Pombal, 1987)	VES	1100–1400	Swamp area	4	LC, LC	AF

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Ololygon flavoguttata (Lutz & Lutz, 1939)	VES,OE	1161	Leaf litter	2	LC, LC	AF
Hylodidae						
Hylodes pipilans Canedo & Pombal, 2007	VES,PF	1134	Stream or rivulet	15	LC, LC	AF, RJ
Megaelosia goeldii (Baumann, 1912)	VES,OE	1134	Puddle in stream	2	LC, LC	AF, RJ
Leptodactylidae						
Leptodactylus latrans (Steffen 1815)	OE	1100–1400	Paved road	1	LC, LC	AF
Microhylidae						
Myersiella microps (Duméril & Bibron, 1841)	VES,PF	1280	Leaf litter	4	LC, LC	AF
Odontophrynidae						
Proceratophrys boiei (Wied-Neuwied, 1824)	VES,PF,OE	1100–1400	Leaf litter	4	LC, LC	AF
Proceratophrys cf. *melanopogon*	VES,PF,OE	1122–1334	Leaf litter	68	LC, LC	AF
SQUAMATA (Lizards)						
Gymnophthalmidae						
Heterodactylus imbricatus Spix, 1825	PF	1161–1278	-	2	LC, LC	AF
Leiosauridae						
Enyalius perditus Jackson, 1978	VES,PF,OE	1153–1278	Tree trunks	11	LC, LC	AF
Urostrophus vautieri Duméril & Bibron, 1837	PF	1334	-	1	LC, LC	
SQUAMATA (Snakes)						
Colubridae						
Chironius bicarinatus (Wied, 1820)	OE	1100–1400	-	1	LC, LC	
Dipsadidae						
Atractus zebrinus (Jan, 1862)	OE	1100–1400	-	2	LC, LC	
Dibernardia affinis (Günther, 1858)	PF	1332	-	1	LC, LC	
Dibernardia persimilis (Cope, 1869)	PF	1334	-	1	LC, LC	AF
Dipsas alternans (Fischer, 1885)	OE	1100–1400	-	3	LC, LC	AF
Dipsas neuwiedi Ihering, 1911	OE	1100–1400	-	2	LC, LC	AF
Dryophylax nattereri (Mikan, 1828)	OE	1100–1400	-	1	LC, LC	

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Echinanthera cephalostriata Di-Bernardo, 1996	OE	1272	Leaf litter	1	LC, LC	
Elapomorphus quinquelineatus (Raddi, 1820)	OE	1100–1400	-	1	LC, LC	AF
Oxyrhopus clathratus Duméril, Bibron & Duméril, 1854	OE	1100–1400		2	LC, LC	AF
Tomodon dorsatus Duméril, Bibron & Duméril, 1854	OE	1100–1400	-	1	LC, LC	
Viperidae						
Bothrops fonsecai Hoge & Belluomini, 1959	OE	1100–1400	-	2	LC, LC	AF
Bothrops jararaca (Wied, 1824)	VES,OE	1100–1400	-	5	LC, LC	AF

The rarefaction curve for amphibian and reptile species did not reach an asymptote and showed confidence intervals with an estimated error of 2.9 species for amphibians and 3.2 species for reptiles (Figure 6). The estimated species richness for amphibians (Jackknife 1 = 23.8 ± 2.1; Chao 1 = 20.1 ± 2.5) was similar to the richness recorded using all the combined sampling methods, including occasional encounters (n = 21 species). For reptiles, however, the estimated richness (Jackknife 1 = 8.8 ± 1.8; Chao 1 = 6.4 ± 2.4) represented approximately half of the total species recorded by all methods, including occasional encounters (n = 16 species).

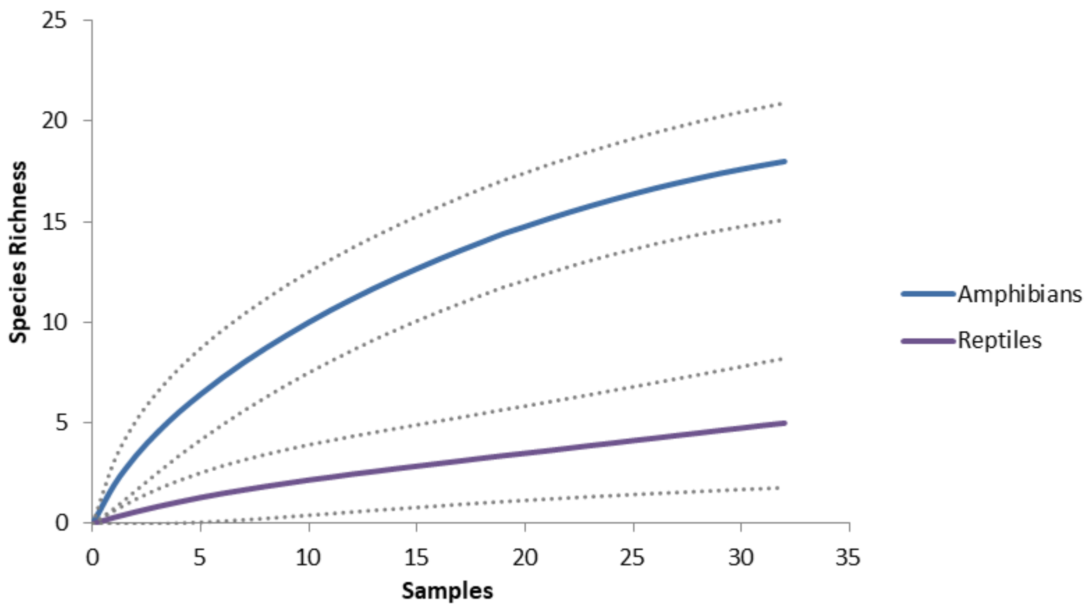

Figure 6. Rarefaction curve for amphibians (blue line) and reptiles (purple line) with 95 % confidence intervals shown by the dotted gray lines, according to the VES and pitfall sampling days at São Lourenço, Nova Friburgo, state of Rio de Janeiro, Brazil.

Anurans accounted for 280 specimens and reptiles for 37 specimens collected mainly in Dense Montane Ombrophilous Forest in the leaf litter and swamp areas, as well as in streams, bromeliads, and open areas. For amphibians, our data recorded one (4,8%) cryptozoic species (*I. octavioi*), one (4,8%) fossorial species (*M. microps*), three (14,3%) rheophilic species (*M. goeldii*, *H. pipilans* and *C. eleutherodactylus*), nine (42,8%) species with different degrees of arboreal habit (*F. fissilis* and all Hylidae species) and seven (33,3%) terricolous habit species (*Ischocnema* sp. (gr. *guentheri*), *I. parva*, *R. ornata*, *R. icterica*, *L. latrans*, *P. boiei*, *Proceratophrys* cf. *melanopogon*).

For lizards, our data recorded one (33,3%) fossorial species (*H. imbricatus*) and two (66,7%) species with different degrees of arboreal habit (*E. perditus* and *U. vautieri*), whereas for snakes, we recorded one (7,7%) fossorial species (*E. quinquelineatus*), five (38,4%) species with different degrees of arboreal habit (*C. bicarinatus*, *D. alternans*, *D. neuwiedi*, *D. nattereri* and *B. jararaca*) and seven (57,9%) terricolous species (*A. zebrinus*, *D. affinis*, *D. persimilis*, *E. cephalostriata*, *O. clathratus*, *T. dorsatus* and *B. fonsecai*). Most species were recorded within Dense Montane Ombrophilous Forest and in surrounding open areas. Within these habitats, several microhabitats were surveyed, with the most common being leaf litter (Brachycephalidae, Bufonidae, Cycloramphidae, Hylidae, Microhylidae, Odontophrynidae, and Dipsadidae), streams (Hylodidae), and swamps (Hylidae) (Table 1). Additional microhabitats included paved roads (Leptodactylidae), bromeliads (Hemiphractidae), and various other water bodies and vegetation types (Hylidae and Hylodidae) (Table 1). The biometric data of the collected specimens are in the Supplementary Material Tables 2 and 3.

Among amphibians, the Hylidae family was the richest (eight species), followed by Brachycephalidae (3), Bufonidae, Hylodidae, and Odontophrynidae (each with two species), and Cycloramphidae, Hemiphractidae, Leptodactylidae, and Microhylidae (each with a single species). Eighteen amphibians are endemic to the Atlantic Forest, three of which are restricted to Rio de Janeiro and typical of medium to high altitudes (Table 1). Regarding squamate reptiles, Dipsadidae is the richest family for snakes (10 species), followed by Viperidae (two species) and Colubridae (with a single species). Leiosauridae was the richest family of lizards (two species), followed by Gymnophtalmidae (with a single species). Nine of these species are endemic to the Atlantic Forest, some of which are typical high-altitude species (*e.g.*, *H. imbricatus*, *A. zebrinus* and *B. fonsecai*) (Table 1). Two venomous snakes of health concern were recorded in the area, *B. jararaca* and *B. fonsecai*. All the species of amphibians and reptiles sampled are classified as least concern in terms of conservation status by both global (IUCN) and national (ICMBio/MMA) conservation agencies (Table 1).

DISCUSSION

Our results represent a valuable contribution to the knowledge of herpetofauna in high-altitude regions of the Atlantic Forest, particularly in the Serra dos Órgãos mountains. We achieved species richness comparable to the Jackknife 1 estimate for amphibians and nearly twice the predicted number of reptile species. The outcome for the reptiles was achieved by the accumulation of new species via complementary methods. The results for amphibians are even more consistent with those estimated by the Chao 1, whereas for reptiles the estimated richness was lower, and therefore closer to that observed using standardized methods. The difference between the observed and the estimated species richness is due to the use of complementary sampling methods, highlighting the importance of combining sampling methods.

The present study employed a sampling effort comparable to that of Siqueira *et al*. (2011) in Parque Estadual dos Três Picos (PETP), where amphibians were surveyed at altitudes of 1,100–1,400 meters using 300 hours of active search effort in Theodoro de Oliveira, a neighborhood of Nova Friburgo located approximately 7 km from our sampling site. Excluding pitfall trap records, we sampled 19 anuran species, whereas Siqueira *et al.* (2011) sampled 31. Nonetheless, we recorded four anuran species not sampled by Siqueira *et al.* (2011) (*H. pipilans*, *M. goeldii*, *L. latrans*, *O. hiemalis*). Although *Ischnocnema parva* was sampled in our study, it was not highly abundant as recorded by Siqueira *et al.* (2011). Notably, some species collected in Theodoro de Oliveira in high abundance and were not found in the present study, such as *Brachycephalus* spp., *Cycloramphus parvulus* and *Hylodes charadranaetes*.

Folly *et al.* (2016) conducted herpetofaunal surveys in the municipality of Teresópolis, within the boundaries of the Serra dos Órgãos National Park (PARNASO), located 43 km from our sampling site, documenting 26 species within the 1,200 – 1,500 m elevation range, 14 of which were identified during sampling and 12 through secondary data. Six species were shared between our study and that of Folly *et al.* (2016): *I. parva*, *R. icterica*, *F. fissilis*, *B. circumdata*, *C. eleutherodactylus*, and *O. albicans*. We also recorded five genera that were not detected in their surveys: *Boana*, *Dendropsophus*, *Megaelosia*, *Leptodactylus*, and *Myersiella*. Conversely, their study identified three genera that were not found in our sampling: *Brachycephalus*, *Gastrotheca*, and *Aplastodiscus*. These differences in species composition may be related to anthropogenic environmental heterogeneity at our sampling site, including grazing areas, eucalyptus plantations, agriculture, and man-made structures. Such features likely contribute to the presence of generalist species with broader niche breadths. The absence of *Brachycephalus* spp. appears atypical, given the high number of individuals recorded in both comparative studies conducted along similar altitudinal gradients. This absence may also be explained by the human-induced alterations to the ecosystem mentioned above (Lima *et al.* 2013). The species *Hylodes charadranaetes* and *H. pipilans* exhibit limited spatial overlap in parts of the municipalities of Teresópolis and Petrópolis, near the borders of the PETP and PARNASO. This may account for the exclusive detection of *H. pipilans* in our study. Additionally, *H. charadranaetes* tends to occur at higher altitudes compared to *H. pipilans* (up to 1.400 m see Weber *et al.* 2008). Nevertheless, our record of *H. pipilans* may represent an altitudinal extreme for the species, approaching the upper range typically occupied by *H. charadranaetes*.

Regarding Squamata, there is no available information in the literature for Parque Estadual dos Três Picos or its surroundings at elevations of 1,000 meters or higher. The most comprehensive reptile inventory for this region is provided by Almeida-Gomes *et al*. (2014), who recorded 37 species at the Reserva Ecológica de Guapiaçu (REGUA), including one amphisbaenian, 10 lizards, 24 snakes, one crocodilian, and one chelonian. However, the highest elevation surveyed in that study was only 700 m, leaving higher-altitude areas, such as those above 1,000 m in Parque Estadual dos Três Picos, without representation in the literature. Although our study had a lower effort compared to Almeida-Gomes *et al*. (2014) (6,600 bucket-days for pitfalls, 2,631 hours for VES and 4,750 m² of forest floor surveyed for quadrat method), we recorded three lizards (*H. imbricatus*, *E. perditus* and *U. vautieri*) and seven snake species (*A. zebrinus*, *D. persimilis*, *D. alternans*, *D. nattereri*, *E. quinquelineatus*, *T. dorsatus* and *B.*

fonsecai) not recorded by them. These comparisons illustrate how high-altitude assemblages in the region can differ from those at lower or even similar elevations, underscoring the need for further studies on reptile communities above 1,000 m.

Ischnonecma sp. (gr. *guentheri*) accounted for 35% of all amphibian specimens sampled. Together with *Proceratrophys* cf. *melanopogon*, *R. icterica*, and *R. ornata*, these species represented 70% of all recorded individuals and 80% of all anuran amphibians. *Ischnocnema* species of the *guentheri* group, *R. icterica* and *R. ornata,* are widely distributed in the Atlantic Forest (Gehara *et al.* 2013; Frost 2025), whereas *Proceratophrys melanopogon* is restricted to Serra do Mar from São Paulo and Rio de Janeiro, found in forest areas above 800 m (Mângia *et al.* 2014). Some ecological studies have shown that a few species tend to be ecologically dominant (Magurran 2004), constituting most individuals in an ecological community (*e.g.*, Dorigo *et al.* 2021). Species of the genus *Ischnocnema* lay eggs on the moist forest floor and exhibit direct development, thus, they do not rely on water bodies for reproduction (Pombal & Haddad 2007). Species with direct development from the family Brachycephalidae appear to be the most common at high elevations in PETP (Siqueira *et al.* 2011) and are dominant members of leaf-litter amphibian communities in Neotropical rainforests (*e.g.*, Dorigo *et al.* 2021). However, some of the dominant species we observed (*e.g., Ischnocnema* gr. *guentheri*, *Proceratophrys* cf. *melanopogon*) may represent cryptic species or species complexes (*e.g.,* Gehara *et al.* 2013). In such cases, abundance patterns may vary, and this information cannot be accurately assessed in studies based solely on morphological identification. For example, *Ischnocnema guentheri* was previously considered a widely distributed species in southern Brazil; however, molecular and acoustic analyses by Gehara *et al.* (2013) revealed that it is, in fact, microendemic to the municipality of Rio de Janeiro. Uncertainty in the identification of individuals belonging to the genera *Ischnocnema* and *Proceratophrys* highlights the need for further taxonomic studies to clarify the species limits within these groups, avoiding interpretation biases.

Enyalius perditus accounted for 30% of all the reptile specimens collected. When combined with specimens of *Bothrops jararaca* and *Dipsas alternans*, these three species represented 51%

of all the reptile records. In contrast, thirteen Squamata species were represented by only one or two individuals. The low recorded abundance of Squamata reptiles in São Lourenço is consistent with the general trend of low encounter rates for this group in the Atlantic Forest, particularly when compared to other Brazilian biomes such as the Amazon, especially at higher elevations, where thermal energy is lower than in lowland regions (*e.g.*, Dixon & Verdade 2006). Species-specific biological traits and habits (*e.g.*, body size, fossorial behavior and arboreality) influence the detectability of reptiles in field surveys, often resulting in lower representation in scientific collections and publications when compared to more easily sampled vertebrate groups such as amphibians, birds, and mammals (Guedes *et al.* 2023b). This pattern is particularly evident in structurally complex environments, such as high-altitude dense forests, further reinforcing the need for targeted biodiversity inventories (*e.g.*, Guedes *et al.* 2020; Guedes *et al.* 2023b).

The São Lourenço study area is home to a variety of species with wide altitudinal ranges (130 - 980 m), such as the amphibians *H. pipilans* (245 - 814 m) reported by Carmo *et al.* (2022), *D. minutus*, *B. faber*, *Ischnocnema* sp. (gr. *guentheri*), *P. boiei*, and *R. icterica*, as previously reported by Araujo *et al.* (2010). Additionally, some species are typically found at high elevations, including *Bokermannohyla circumdata* (500 - 980 m) and *P. melanopogon* (800 - 1480 m), as well as the snake *B. fonsecai* (1000 - 1600 m), and the lizards *U. vautieri* (1440 m) and *H. imbricatus* (1250 m) (Campbell *et al.* 2004; Araujo *et al.* 2010; Novelli *et al.* 2011; Mângia *et al.* 2014; Entringer *et al.* 2022).

Approximately 70% of the species recorded in our study are endemic to the Atlantic Forest (Tozetti *et al.* 2017), for which we provide valuable data on habitat use and altitudinal distribution. Notable findings also include the presence of the anurans *Ololygon albicans and Hylodes pipilans*, which are endemic to Rio de Janeiro state, and *Cycloramphus eleutherodactylus,* which, together with *H. pipilans*, lacks global assessment data (IUCN 2024). Additionally, we encountered typical forest species such as the amphibians *M. microps*, *P.* cf. *melanopogon*, and *I. octavioi* (Dixon & Verdade 2006); the snake *B. fonsecai*, typical of altitude with few records in the Rio de Janeiro state (Campbell *et al*. 2004); the lizards *U. vautieri, H. imbricatus*, which are rare species with low detectability, mainly the last one due to its fossorial habit (Zocca *et al.* 2023); and *E. perditus*, a species sensitive to fragmentation and deforestation (Barreto-Lima *et al.* 2013).

Finally, most fossorial and secretive amphibian and reptile species in our study were recorded using pitfall traps. Therefore, increasing the pitfall sampling effort in the area could further improve our understanding of the species associated with high-altitude environments and leaf litter (see appendix S4). Given the limited literature on herpetofauna, particularly the Squamata reptiles, in the Atlantic Forest at elevations above 1,000 m, our findings highlight the importance of fieldwork studies — even short-term ones — in helping to fill this knowledge gap (*e.g.*, Araujo *et al.* 2010). The study area lies within the boundaries of the PETP buffer zone and constitutes a portion of its adjacent forest. Although conserved, this area is bordered mainly by pasture for livestock farming, which leads us to the following recommendations: i) conserve forest remnants by restoring degraded areas with native forest and replacing the existing exotic eucalypt plantations whenever possible. An alternative to safeguard the researched area amid land use change is to incorporate it inside the boundaries of the PETP; ii) ensure the cleanliness of water sources, which are vital for human communities and serve as habitats for many species, especially anuran species that depend on running waters such as *Cycloramphus* spp., *Megaloesia* spp., *Hylodes* spp.; iii) promote sustainable recreational and environmental education activities for the local community; and iv) provide infrastructure, researcher housing, and other facilities for scientific research activities, fieldwork courses, and sustainable tourism. Additionally, to improve the understanding of the groups in the region, future studies should focus on identifying cryptic species detected through molecular techniques, alongside ecological investigations examining the relationships between forest patch size and species richness, as well as the effects of climate change on their populations.

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